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Biological Control 34 (2005) 180–186
Biological Control
www.elsevier.com/locate/ybcon

Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae) ☆

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Received 31 January 2005; accepted 13 May 2005

Abstract

Entomopathogenic fungi are often considered to be efficacious only with high ambient moisture. When adult female *Rhyzopertha dominica* were introduced onto wheat kernels with 200 mg/kg of *Beauveria bassiana* and/or 100 mg/kg of diatomaceous earth (DE) and incubated at 26, 30, 32.5, or 34 °C, there was significantly lower emergence of adult progeny at 43% RH than at 75% RH. On grain that was treated with both the fungus and DE, there were 95–97% reductions in adult progeny vs. controls at 43% RH and 22–91% reductions at 75% RH. On grain that was treated with fungus alone, there were 82–90% reductions in progeny adults at 43% RH and only 8–76% reductions at 75% RH. When adult beetles were exposed to *B. bassiana* at 30 °C and RHs of 43, 56, 75, or 85%, the mortality means were not significantly different among humidities, but when the beetles were exposed to *B. bassiana* from egg to adult under the same regimes, the number that survived was lower for *B. bassiana*-treated beetles than for control beetles at 43, 56, or 85% RH, but did not differ significantly at 75% RH. A temperature of 34 °C had a negative impact on efficacy at 75% RH, but not at 43% RH. Preincubation of conidia at 30 °C and 75% RH, but not 43% RH, for 5 days resulted in reduced mortality of adult beetles. The germination rates of *B. bassiana* conidia declined more rapidly at the greater RHs and when mixed with wheat than when unmixed, thereby reducing residual activity. The lower progeny adult production at 43% RH than at 75% RH, suggests that stress contributes to the greater fungus and DE effects at the lower humidity.
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Keywords: *Beauveria bassiana*; *Rhyzopertha dominica*; Diatomaceous earth; Lesser grain borer; Desiccation; Temperature; Stress

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is a major cosmopolitan pest of stored wheat. *Beauveria bassiana* (Balsamo) Vuillemin has shown potential for controlling *R. dominica*, especially in combination with desiccant dusts (Lord, 2001). A perception that its efficacy is dependent on environments of high ambient moisture is an impediment to its adoption for stored-product pest management. The validity of that perception is unclear. The reported influence of ambient moisture on *B. bassiana* infectivity for insects varies from no effect (e.g., Ferron, 1977; Marcandier and Khachatourians, 1987) to a direct correlation between moisture and efficacy (e.g., Haraprasad et al., 2001; Ramoska, 1984). Similarly, temperature effects on *B.*

☆ This article reports the results of research only. Mention of a proprietary product does not constitute a recommendation or endorsement by the U.S. Department of Agriculture.

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1049-9644/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2005.05.004

bassiana's infectivity, development, and survival are key components of its use for insect control and have been the subject of many studies.

Temperature and humidity conditions in the stored grain environment fluctuate within a relatively narrow range when the grain mass is large, but grain storage temperatures often exceed the temperature optima for entomopathogenic fungi. Warm season temperatures in the range of 27–34 °C, which coincide with maximum insect activity, have been recorded in the top meter of farm-stored wheat in Kansas (Hagstrum, 1987). The upper end of that range is near the upper limit for growth of many *B. bassiana* isolates, including the commercial isolate used in this study, GHA (Fargues et al., 1997). Accordingly, a key determinant of *B. bassiana*'s potential for pest management in grain is the temperature limits for efficacy against the primary target pests. Grain moisture is adjusted to compromise between loss of profit from low test weight and loss of quality to fungal and insect infestation. The moisture content of stored wheat in North America is mainly in the range of 10.5–13.5% (Hagstrum, 1987; Reed and Pan, 2000), which corresponds to ca. 45–60% RH at 25 °C in hard red winter wheat.

To address concerns about *B. bassiana*'s efficacy in stored grain under operational conditions and to better develop strategies for its use, laboratory assays were conducted with *R. dominica* under selected temperature and humidity conditions and in combination with insecticidal desiccant dust.

2. Materials and methods

2.1. Insects and treatments

The *R. dominica* were from a colony that has been maintained at the USDA Grain Marketing and Production Research Center, Manhattan, KS, for several years and has eastern Kansas origin. Unformulated *B. bassiana* strain GHA was obtained from Mycotech Corporation, Butte, MT. The stock contained 6.3×10^{10} *B. bassiana* conidia per gram. The germination rate of the stock conidia was determined periodically through completion of the experiments. Conidia were spread on Sabouraud dextrose agar (SDA) and incubated for 18 h at 26 °C, and 200 conidia were scored for the presence of visible germ tubes. The germination rate was at least 93% for all assays. The use rate was 200 mg of conidia/kg of wheat kernels in all assays. The diatomaceous earth (DE) was formulated with 10% silica gel (Protect-It, Hedley Technologies, Blaine, WA). It was used at a rate of 100 mg/kg of wheat. Hard red winter wheat was used throughout and 10% was crimped to facilitate feeding by neonates.

2.2. *Rhyzopertha dominica* progeny production when exposed to *B. bassiana* and DE under different temperature and humidity regimes

The initial experiment was a test of temperature and humidity effects on the efficacy of *B. bassiana* and diatomaceous earth individually and in combination. On the second day after adult emergence, female *R. dominica* were selected by exposing ovipositors when applying pressure to the abdomen. Males mate aggressively (Crombie, 1941), and insemination was assumed to be complete. They were held on whole kernel wheat at 30 ± 1 °C and 57 ± 5% RH for 1–2 weeks before use. Each experimental unit consisted of 12 randomly selected females in 118 ml (4 oz) wide-mouth glass jars with 100 g of wheat and filter paper inserted into the lid rims. The wheat was untreated or treated with conidia, DE, or both conidia and DE. Incubation was at 43 ± 1% RH and 26, 30, 32.5, or 34 ± 1 °C, corresponding to vapor pressure deficits of 1.95, 2.41, 2.82, and 2.97 kPa, respectively or at 75 ± 1% RH and 26, 30, 32.5, or 34 ± 1 °C, corresponding to vapor pressure deficits of 0.87, 1.07, 1.11, and 1.32 kPa, respectively. Treatment vessels were placed on grids in $15 \times 30 \times 50$ cm incubation boxes with substratums of saturated K_2CO_3 or NaCl for 43 and 75% RH, respectively (Greenspan, 1977). The grain was conditioned before the assays by incubation for several weeks over the appropriate salt solution until the moisture contents had stabilized within 0.1%. The parental females were removed from the jars after 4 days of incubation. Thus, the data indicate effects on progeny production from oviposition through emergence. The number of progeny that survived to adulthood was scored weekly beginning at 6 weeks post-treatment until emergence ceased. There were four temporal replicates that were set up at intervals of at least 1 week with different *R. dominica* cohorts. Temperature and humidity in the head space of incubation boxes were monitored with HOBO data loggers (Onset, Pocasset, MA).

2.3. Effect of humidity on *B. bassiana* efficacy for *R. dominica* larvae and adults

The assay vessels were as described above but with 50 g of wheat kernels whose moisture content was equilibrated over K_2CO_3 for 43% RH, NaBr for 56% RH, NaCl for 75% RH or KCl for 85% RH (Greenspan, 1977). The respective corresponding wheat moisture contents were 10.9–11.1, 11.7–11.9, 14.3, and 16.2–16.4 and vapor pressure deficits were 2.41, 2.33, 1.07, and 0.68 kPa. The wheat moisture contents were measured with Dickey–John GAC 2000 grain moisture meter. For larval assays, 20 eggs, 3 days post-oviposition, were placed in each vessel and scored for adult emergence after 6 weeks, allowing completion under the test conditions. Assayed adults were of mixed-sexes and aged 1–2 weeks post-emergence. Twenty adults were placed in each vessel, and mortality was scored after 8 days of incubation. There were three assay vessels per replicate and five temporal replicates with separate beetle cohorts for both experiments. Incubation was at 30 ± 1 °C.

2.4. *Beauveria bassiana residual activity on wheat at two humidities*

An assay was carried out to determine the effect of previous incubation on wheat on the efficacy of *B. bassiana* conidia. In this case, scintillation vials with cotton batting closures and 10 g of wheat, with or without conidia were used. Twenty adult *R. dominica* were introduced into each vial at 0, 2, 5, or 9 weeks of preincubation at 30 ± 1 °C and 43 ± 1% RH or 75 ± 1% RH. Mortality was scored after 8 days of incubation. There were three replicates per treatment.

2.5. *Conidia survival*

Conidia were mixed in moisture-equilibrated wheat at the rate of 200 mg/kg and 10 g were placed in each of 27 scintillation vials. For comparison, ca. 10 mg of conidia were placed in vials without wheat. The vials were incubated at 30 ± 1 °C and 43, 56, 75, or 85% RH as described above. Three vials per treatment were destructively sampled at each scoring date. To assess germination rates, samples were suspended in 0.1% Silwet L-77 (Loveland, Greeley, CO), plated on SDA, and incubated for 18 h at 26 °C before counting 100 conidia per vial. From the fourth week when the 18 h germination rates had declined, additional SDA plates with 10 mg/L benomyl to arrest hyphal growth were included and incubated for 42 h to detect slow-germinating but viable conidia. Scoring was done weekly for 7 weeks and on weeks 9 and 12.

2.6. *Statistical analysis*

The data were subjected to ANOVA with Statgraphics Plus software (Manugistics, Rockville, MD) after square root transformation and means were compared with Student–Neuman–Keuls test, except where noted. Conidia survival was further analyzed by probit analysis with Polo Plus (LeOra Software, Berkeley, CA).

3. Results

3.1. *Rhyzopertha dominica progeny production when exposed to B. bassiana and DE under different temperature and humidity regimes*

Both temperature and moisture affected the number of emerging adult progeny for all grain treatments (Fig. 1). The most surprising result of the initial experiment was that, for beetles exposed to *B. bassiana* alone, there were significantly fewer emerging adult progeny at 43% RH than 75% RH at each temperatures with

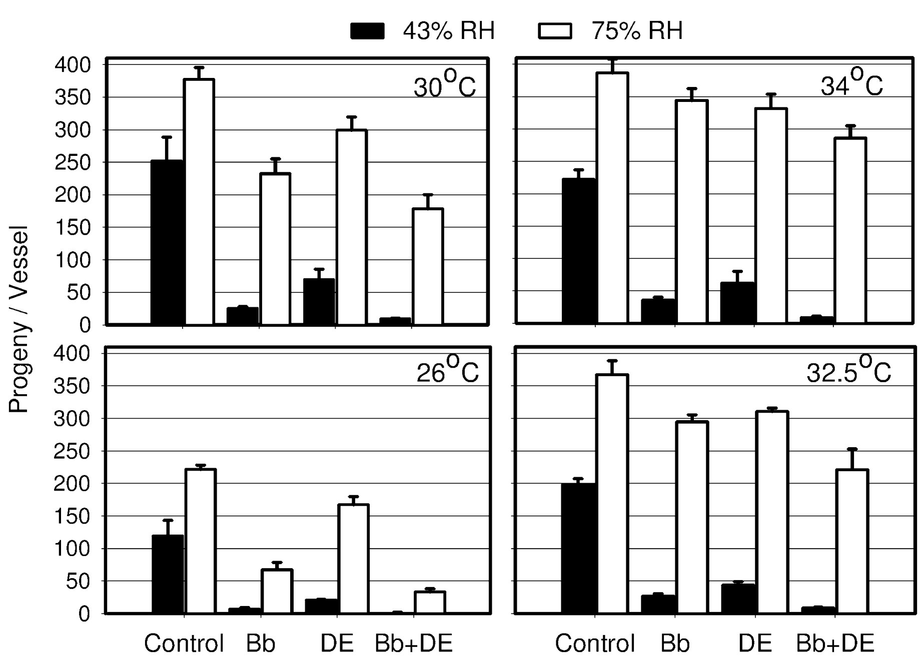

Fig. 1. Adult surviving progeny/vessel of *R. dominica* females exposed to 200 mg/ kg of *B. bassiana* (Bb), 100 mg/kg desiccant dust (DE), *B. bassiana* and desiccant dust (Bb + DE) or no treatment on wheat kernels at four temperatures and either 43% RH (black bars) or 75% RH (open bars). Vessels had 12 parental females ovipositing for 4 days. Bars are standard errors.

$P < 0.01$ ($26 \pm 1\,°C$, $t = 7.2$, $df = 6$; $30 \pm 1\,°C$, $t = 12.5$, $df = 6$; $32.5 \pm 1\,°C$, $t = 24.8$, $df = 6$; $34 \pm 1\,°C$, $t = 20.3$, $df = 6$). With fungus alone, there were 82–90% reductions in progeny adults at 43% RH and only 8–76% reductions at 75% RH. Correspondingly, when the beetles were exposed to *B. bassiana* with DE, there were significantly fewer emerging adult progeny 43% RH than at 75% RH at each temperature with $P < 0.01$ ($26 \pm 1\,°C$, $t = 11.4$, $df = 6$; $30 \pm 1\,°C$, $t = 11.6$, $df = 6$; $32.5 \pm 1\,°C$, $t = 10.2$, $df = 6$; $34 \pm 1\,°C$, $t = 21.5$, $df = 6$). With fungus and DE, there were 95–97% reductions in adult progeny vs. controls at 43% RH and 22–91% reductions at 75% RH. Overall, only DE with *B. bassiana* resulted in significantly fewer emerging adult progeny than the DE alone or control, but it did not differ significantly from *B. bassiana* alone ($F = 12.6$; $df = 3, 123$; $P < 0.01$).

The lowest temperature, 26 °C, was the least favorable to beetle production and the most favorable for treatment efficacy. Adult progeny emergence terminated in 11 weeks at 26 °C and 7 weeks at the higher temperatures. There were significantly fewer untreated progeny adults at 26 °C than at the other temperatures for both 43% RH ($F = 12.4$; $df = 3, 12$; $P < 0.01$) and 75% RH ($F = 19.0$; $df = 3, 12$; $P < 0.01$). When analyzed by percent reduction compared with controls at 43% RH, *B. bassiana* alone had significantly greater effect at 26 °C than at 32.5 or 34 °C ($F = 7.2$; $df = 3, 12$; $P < 0.05$), while for *B. bassiana* with DE there was significantly greater reduction at 26 °C when compared with 32.5 °C only ($F = 6.5$; $df = 3, 12$; $P < 0.01$). For 75% RH, there was significantly greater reduction at 26 °C than other test temperatures for both *B. bassiana* alone ($F = 33.1$; $df = 3, 12$; $P < 0.01$) and *B. bassiana* with DE ($F = 20.3$; $df = 3, 12$; $P < 0.05$). There was no significant temperature effect on reduction of progeny adults versus controls for DE at either 43% RH ($F = 0.9$; $df = 3, 12$; $P = 0.46$) or 75% ($F = 2.2$; $df = 3, 12$; $P = 0.14$). The numerically greatest reduction in F1 survivorship compared with controls was 98.8% with *B. bassiana* and DE at 26 °C and 43% RH.

3.2. Effect of humidity on B. bassiana efficacy for R. dominica larvae and adults

When adults beetles were exposed to *B. bassiana* at 30 °C and relative humidities of 43, 56, 75, or 85%, the respective mortality means of 67.0, 62.0, 56.3, and 65.7% were not significantly different ($F = 1.09$, $df = 3, 16$, $P = 0.38$). There was no control mortality in the adult test. When the beetles were exposed to *B. bassiana* from egg to adult under the same regimes, the number that survived to the adult stage was lower for *B. bassiana*-treated beetles than for control beetles at 43% RH ($t = 8.8$, $df = 4$, $P < 0.01$), 56% RH ($t = 19.1$, $df = 4$, $P < 0.01$), and 85% RH ($t = 4.0$, $df = 4$, $P = 0.016$), but did not differ significantly at 75% RH ($t = 2.7$, $df = 4$, $P = 0.052$) (Fig. 2).

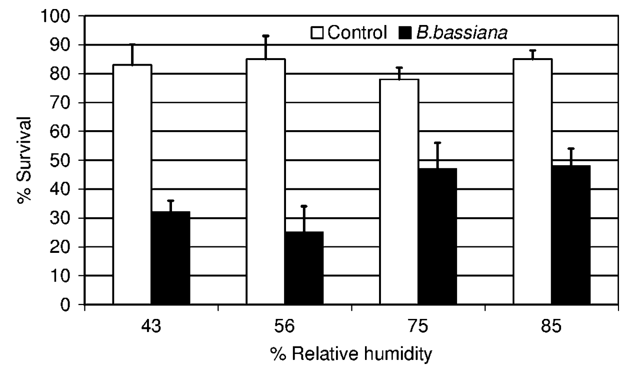

Fig. 2. *Rhyzopertha dominica* survival from egg to adult with exposure to *B. bassiana* at four humidities.

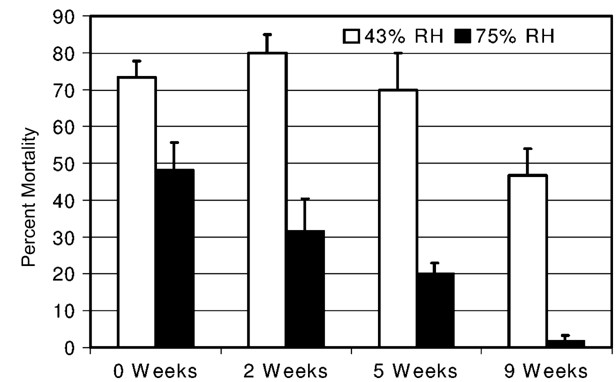

Fig. 3. Mortality of *R. dominica* adults exposed to *B. bassiana* conidia that had been preincubated with wheat kernels at two RHs for different time periods. There was no control mortality.

3.3. Beauveria bassiana residual activity on wheat at two humidities

When adult beetles were exposed to conidia on wheat without preincubation of conidia, mortality was significantly greater at 43 than 75% ($t = 2.9$, $df = 4$, $P = 0.04$) (Fig. 3). Only after 9 weeks of conidia preincubation at 43% RH, was the mortality significantly different from conidia that were not preincubated ($F = 8.4$; $df = 3, 8$; $P < 0.01$), but the mortality with conidia that were preincubated at 75% RH for 5 weeks was significantly lower than for conidia that were not preincubated ($F = 10.9$; $df = 3, 8$; $P < 0.05$). In addition, the mortality was significantly lower for 75 than 43% RH with preincubation of 2 weeks ($t = 4.7$, $df = 4$, $P < 0.01$), 5 weeks ($t = 4.8$, $df = 4$, $P < 0.01$), or 9 weeks ($t = 6.0$, $df = 4$, $P < 0.01$). There was no control mortality.

3.4. Conidia

Both humidity and substrate had significant effects on the germination rates of conidia (Fig. 4). When compared after 1 week of incubation with wheat kernels, germination rates varied significantly ($F = 17.2$; $df = 4, 10$; $P = 0.0002$), and only the rate for conidia held at 56%

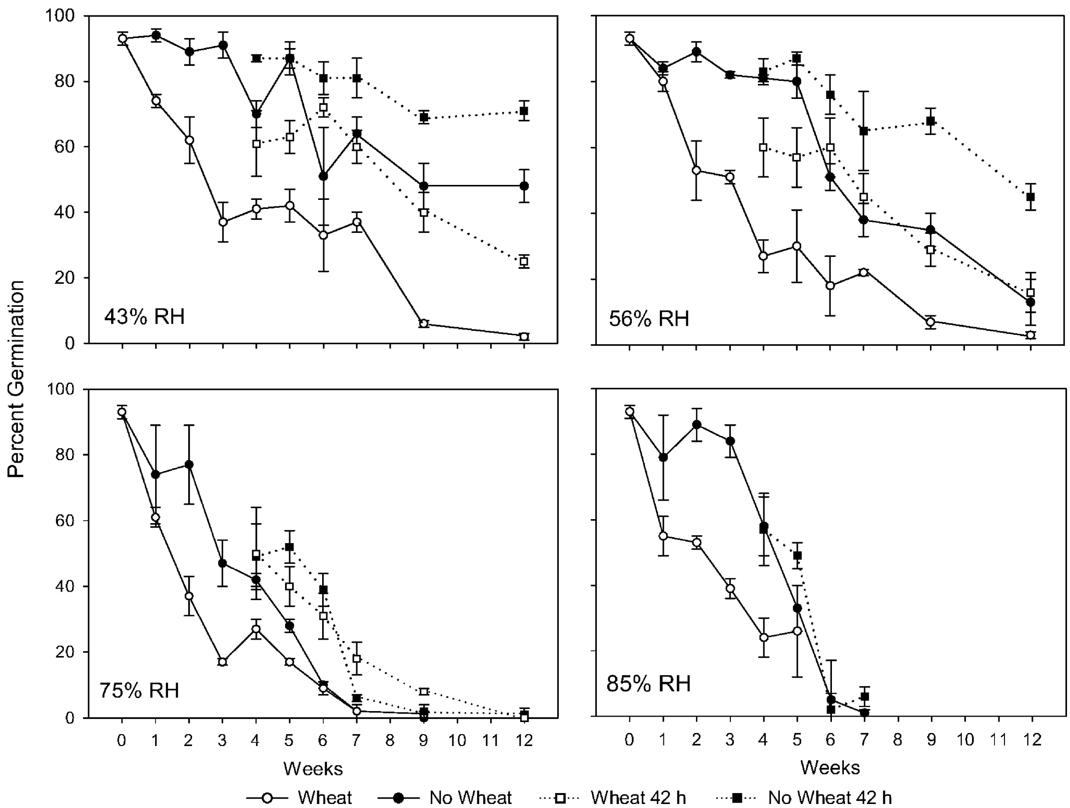

Fig. 4. Percent *B. bassiana* 18 h germination over time of incubation with or without wheat kernels. Forty-two hours germination is from medium with benomyl.

RH was not significantly lower by Dunnett's test with $P > 0.05$. Median numbers of weeks to loss of the ability to germinate in 18 h (±95% CI) for conidia mixed with wheat were 3.2 (1.4–4.6) at 43% RH, 2.7 (2.0–3.3) at 56% RH, 1.6 (1.1–1.9) at 75% RH, and 1.8 (0.8–2.6) at 85% RH. Even at 43% RH, where germination rates declined most slowly, the rates were significantly greater for conidia that were not mixed with wheat ($t = 8.5$, $df = 4$, $P < 0.01$). Greater germination rates were observed after 42 h on the SDA with benomyl, demonstrating that speed of germination was reduced, thereby giving the appearance of more rapid loss of viability than actually occurred. Wheat that was incubated at 85% RH was subject to infestation by *Aspergillus* spp. and *Penicillium* spp. and was impractical to score *B. bassiana* survival after 5 weeks.

4. Discussion

I previously reported that desiccant dusts, which cause water loss by cuticular abrasion and loss of surface lipids, synergize *B. bassiana* for control of *R. dominica* (Lord, 2001). This report confirms that the combination of DE and *B. bassiana* is more effective than either of the treatments alone over a range of environmental conditions that correspond to those of the target's habitat (Birch, 1943a,b, 1953).

The effect of ambient moisture on the efficacy of fungi in controlling insects is a complex and controversial matter. Among stored-product insects for example, Searle and Doberski (1984) reported that *B. bassiana* infected *Oryzaephilus surinamensis* (L.) at 100% RH but not at 90% RH, while Akbar et al. (2004) reported no statistically significant differences in the mortality of larval *Tribolium castaneum* (Herbst) after exposure to the same fungus at 54 or 75% RH. Brower et al. (1995) state that fungi have not been developed as microbial control agents of stored-product pests "because of their dependency upon high ambient humidities" and poor understanding of virulence factors. While the latter premise is legitimate, the data presented here rebut the former. Indeed, the greatest *B. bassiana* efficacy for *R. dominica* was at the lowest RH tested, 43%.

There is a trend in some specialties toward the use of vapor pressure deficit as a measure of the evaporative capacity of air, presumably because it is adjusted for temperature. Relative humidity and temperature (associated vapor pressure deficits cited in the Section 2) are used in this discussion in part because they are the measures used in the literature that relates moisture to the biology of the lesser grain borer and other grain insects and are meaningful to workers in the area.

A great deal more study will be needed to fully understand the reasons for these results, but some speculation is warranted. Lesser grain borer survival from egg to adult is less than 50% at a moisture content of 11% and optimal temperatures (Birch, 1943a). The grain that was equilibrated at 43% RH in this study was 10.9–11.1% moisture. This is a moisture content that is unfavorable for *R. dominica* (Birch, 1943a, 1953) and presumably is a stress on the beetles. Although in this study, survival from egg to adult was not affected by the moisture contents at 30 °C, Khare and Agrawal (1970) reported higher mortality during the development at 30 °C of lesser grain borers reared on wheat at 50 or 60% RH than at 70 or 80% RH. Longstaff (1999) reported that *R. dominica*'s development rate decreased with decreasing moisture. While the stress is a logical suspect as the cause of increasing susceptibility to the fungus, it is a very broad concept and numerous stress-related physiological changes may come into play. Desiccation stress may cause changes in the cuticular chemistry that affect the ability of conidia to attach, germinate, and penetrate. It may compromise cellular and humoral defenses. It may even alter behavior in a manner that favors fungal infection. Desiccation induces alteration in the cuticular lipid composition of other stored-product beetles (Howard et al., 1995), and almost certainly does so in *R. dominica*. Considering the importance of cuticular lipids in the conidial attachment (Boucias et al., 1988; Lord and Howard, 2004) and germination (James et al., 2004; Smith and Grula, 1982), their alteration under desiccation stress would certainly affect susceptibility to entomogenous fungi.

The optimum temperature range for *R. dominica* development is reported to be 32–35 °C with a minimum of temperature of 23 °C and a minimum RH of 40% (Birch, 1953). Lesser grain borer females oviposit preferentially on wheat of ca. 14% moisture (70% RH) and rarely on wheat of less than 11% moisture (Birch, 1943b). The most stressful of the environments that were included in the adult progeny production experiment, 43% RH and 26 °C is within the range of conditions that the lesser grain borer encounters in nature and corresponded to the greatest *B. bassiana* efficacy. The data for that experiment included an oviposition component. Oviposition may have been reduced by temperature and moisture stress. But when the beetles were exposed to *B. bassiana* in the egg stage, significantly reduced survival as compared with controls occurred only with the lower moisture levels, supporting the case that stress is a key factor in the fungus effect.

The results presented here are not at variance with the concept of a moisture requirement for conidial germination and fungus efficacy. When *R. dominica* was exposed to *B. bassiana* from egg to adult at four humidities, the mortality was not significantly different from that of the controls at 75% RH but was at 85% RH. There is no doubt that moisture enhances efficacy, but it is only one factor in a complex phenomenon.

Rhyzopertha dominica eggs hatch in 4–9 days, and larvae penetrate grain soon after hatching. The young larvae enter only damaged kernels (Howe, 1950), but abrasion made by the adults ensure that entry points are readily available (Schwardt, 1933). The first instar can last from 4 days to more than 20 days (Howe, 1950), and how much of that time is external is not known. The beetles feed internally within a single kernel through adult emergence. Consequently, adults and young larvae are the stages that are vulnerable to fungus exposure. Two findings of this study indicate that both *B. bassiana*'s efficacy and its interaction with humidity levels are greatest for young larvae. The mortalities were moderate and not significantly different among humidities when the beetles were exposed to *B. bassiana* as adults, and the moisture effect was significant when they were exposed as eggs.

It is well established that survival of entomogenous Hyphomycetes conidia is favored by dry conditions (Wraight et al., 2001). The confirmation of that phenomenon herein is not surprising, but it has implications for the use of *B. bassiana* in stored products. Given the low slope of dose mortality curves for *R. dominica* treatment with *B. bassiana* (Lord, 2001), conidial survival is likely to have only a minor effect during the time frame of these experiments. Lord (2001) reported that a 3-fold increase in *B. bassiana* dose did not significantly increase mortality of *R. dominica* larvae. On the other hand, it would determine the duration of residual activity. The effect on residual activity is confirmed by the mortality results for adult *R. dominica* exposure to *B. bassiana* after the conidia had been preincubated at 43 or 75% RH.

An interesting, but perhaps not surprising, finding was less conidial survival on wheat substrate than in vials without wheat. Plants have evolved an array of defenses against fungi that are pathogenic for them, and those defenses would certainly affect entomopathogenic fungi. In addition, wheat surface moisture may be slightly greater than that of the contact surfaces without wheat. While the result is a clear indication of a negative effect of the kernel substrate on conidial germination, it is a conservative one. No doubt a substantial proportion of the conidia were in clumps and not in direct contact with the kernels.

In summary, it is demonstrated here that low ambient moisture can be an advantage rather than an impediment to *B. bassiana* efficacy for *R. dominica*. The advantage is increased by combination with a desiccant dust. In addition, the results demonstrate that *B. bassiana* is efficacious within the usual operative temperatures of stored grain.

Acknowledgments

I am grateful to Thomas Morgan for able technical assistance and to Drs. Robert Everich and Michael

Mullen for helpful comments on an earlier draft of the manuscript.

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